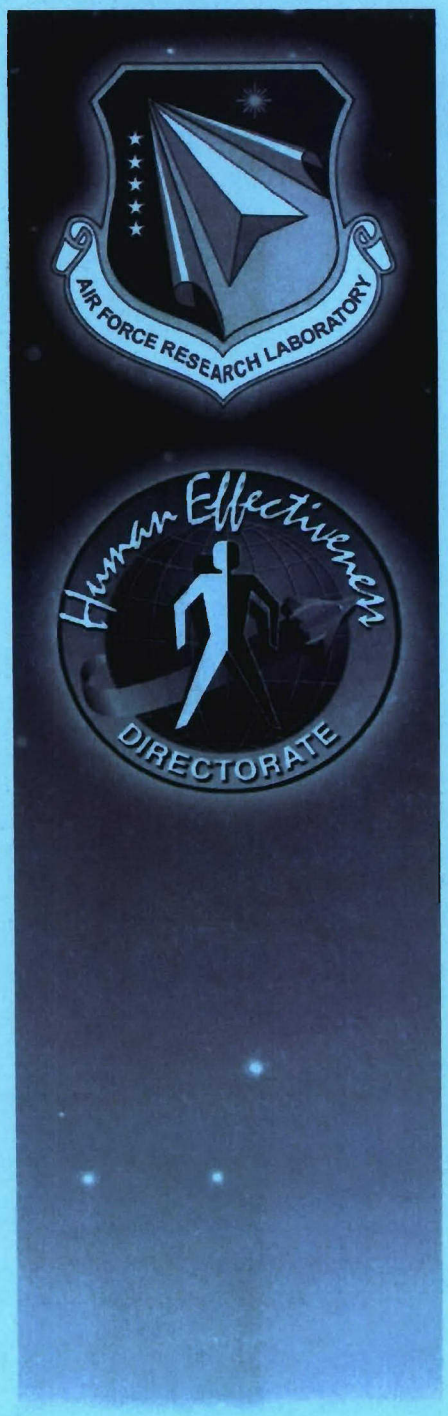
AFRL-HE-BR-TR-2006-0044



Air Force Research Laboratory

INJURY THRESHOLDS FOR TOPICAL CREAM-COATED SKIN OF HAIRLESS GUINEA PIGS (CAVIA PORCELLUS) IN NEAR INFRARED REGION

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JUNE 2006

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

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Form Approved
OMB No. 0704-0188

REPORT DOCUMENTATION PAGE

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
31 JANUARY 2006	Technical Report	November 2005-December 2006

4. TITLE AND SUBTITLE

Injury Thresholds for Topical Cream-Coated Skin of Hairless Guinea Pigs (Cavia Porcellus) in the Near Infrared Region

5a. CONTRACT NUMBER
F41624-02-D-7003 (0009)

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER
7757

5e. TASK NUMBER
B2

5f. WORK UNIT NUMBER
26

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Air Force Research Laboratory
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2650 Louis Bauer Dr.
Brooks City-Base, TX 78235

10. SPONSOR/MONITOR'S ACRONYM(S)
AFRL/HEDO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-HE-BR-TR-2006-0044

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; distribution unlimited.

13. SUPPLEMENTARY NOTES
Contract Monitor – Maj Lawrence Schad

14. ABSTRACT

The reflectance and absorption of the skin plays a vital role in determining how much radiation will be absorbed by human tissue. Any substance covering the skin would change the way radiation is reflected and absorbed and thus the extent of thermal injury. Hairless guinea pigs (*cavia porcellus*) in vivo were used to evaluate how the minimum visible lesion threshold for single-pulse laser exposure is changed with a topical agent applied to the skin. The ED_{50} for visible lesions due to an Er: glass laser at 1540 nm with a pulse width of 50 ns was determined, and the results were then compared to the Takata skin model. The ED_{50} is compared with the damage threshold of skin coated with a highly absorbing topical cream at 1540 nm to determine its effect on damage pathology and threshold. The ED_{50} for the guinea pig was then compared to similar studies using Yucatan minipigs and Yorkshire pigs at 1540 nm and nanosecond pulse duration [1, 2]. The damage threshold at 24 hours of a Yorkshire pig for a 2.5-3.5 mm spot size diameter beam for 100 ns was 3.2 Jcm^{-2} was very similar to our ED_{50} of 3.00 Jcm^{-2} for the hairless guinea pigs.

15. SUBJECT TER MS

laser, laser safety, skin, Takata skin model, MVL, ED_{50}, guinea pigs, laser induced breakdown

16. SECURITY CLASSIFICATION OF: UNCLASSIFIED			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Ginger M. Pocock
Unclas	Unclas	Unclas	U	29	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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ABSTRACT

The reflectance and absorption of the skin plays a vital role in determining how much radiation will be absorbed by human tissue. Any substance covering the skin would change the way radiation is reflected and absorbed and thus the extent of thermal injury. Hairless guinea pigs (*cavia porcellus*) in vivo were used to evaluate how the minimum visible lesion threshold for single-pulse laser exposure is changed with a topical agent applied to the skin. The ED_{50} for visible lesions due to an Er: glass laser at 1540 nm with a pulse width of 50 ns was determined, and the results were then compared to the Takata skin model. The ED_{50} is compared with the damage threshold of skin coated with a highly absorbing topical cream at 1540 nm to determine its effect on damage pathology and threshold. The ED_{50} for the guinea pig was then compared to similar studies using Yucatan minipigs and Yorkshire pigs at 1540 nm and nanosecond pulse duration [1, 2]. The damage threshold at 24 hours of a Yorkshire pig for a 2.5-3.5 mm spot size diameter beam for 100 ns was 3.2 Jcm^{-2} was very similar to our ED_{50} of 3.00 Jcm^{-2} for the hairless guinea pigs.

1. INTRODUCTION

As the skin is the largest organ of the body, the probability of tissue exposure from optical radiation is far more likely for the skin than that for the eye. Injury to large areas of skin is a significant incident since these injuries may lead to serious loss of bodily fluids, toxemia, and systemic infections. Yet there is limited research compared to laser eye injury for a protection factor for the skin against lasers. Laser radiation injury to the skin is comparable to the eye except in the retinal hazard region (400-1400 nm). The most damaging wavelengths for skin have been lasers operating in the near infrared and infrared range which penetrate the skin into the subcutaneous tissue causing deep thermal injury [3]. Many of these lasers are used in military settings and are capable of producing high peak power with short pulses [4]. This type of exposure has proved to cause more extensive damage than continuous wave lasers. The reflectance of the skin plays a vital role in determining how much radiation will be absorbed. Any substance covering the skin would change the way radiation is reflected and absorbed and thus the extent of thermal injury. In this study, the effective dose required to produce an observable response 50% of the time, also known as the ED_{50}, was determined for hairless guinea pigs (in vivo) at 1540 nm using 42-65 ns pulses. In addition, these results are then used to evaluate how the minimum visible threshold for single pulse nanosecond laser exposure is changed with the addition of a covering agent on the surface of the skin. Similar studies have been done using modeling to demonstrate contact thermal burns and temperature profile in skin cover for competitive estimation of heat protection properties of materials [5]. Utilization of many regions of the electromagnetic spectrum may warrant a model of possible protection factors against some wavelengths.

The hairless guinea pig has skin which is physiologically similar to humans and has the added advantage that depilation is not required prior to every procedure [6]. The guinea pigs did not show any kind of visible damage after the covering agent was applied one hour and twenty four hours later as a result of plasma shielding. The ED_{50} is compared to

a similar study done using Yucatan minipigs [2]. The Yorkshire and Yucatan mini-pig are commonly used as in vivo skin models for damage threshold determination for national laser safety standards used in the ANSI Z.136.1 [7]. Of the two, the Yucatan mini-pig has been deemed the more applicable animal model for laser-induced skin injury investigations. A comparison of skin thickness between the Yucatan mini-pig and the arms, neck, and face of human skin are statistically identical [8]. The hairless guinea pig epidermis is of similar thickness to that of human skin with distinct strata, serrated/non serrated basil keratinocytes and shallow dermal papillae [6].

2. MATERIALS AND METHODS

2.1 Subjects

A total of (3) male hairless guinea pig was used for all ED_{50} exposures and (1) was used to test the covering agent. The hairless breed was chosen because of its similarity to human skin and because depilation is not required. The guinea pigs were procured from Charles River Laboratories, Wilmington, MA. All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals under a protocol approved by the Brooks City-Base, TX Institutional Animal Care and Use Committee (IACUC) [9, 10]. Each guinea pig weighed from 550 to 720 grams and was between 3 and 6 months of age. The guinea pigs were fed commercially available diets and had unlimited access to water. Twelve hours prior to procedure, solid food was withheld. The animals involved in this study were procured, maintained, and used in accordance with the Federal Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources --National Research Council [9]. Brooks City-Base, TX has been fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) since 1967.

Animals were anesthetized with a single injection of xylazine (5 mg/kg of body weight) intramuscular (IM) and ketamine (40 mg/kg) IM. After sedation, the guinea pig's skin was cleansed to remove any debris on the skin surface. The cleansed skin was inspected and photographed to make sure scratches or any other irritations that existed prior to the procedure were noted. Pulse rate was monitored using a reflectance pulse oximeter on the foot. The animal's internal temperature was monitored using a rectal digital thermometer and maintained using a heated water blanket throughout all of the procedure.

2.2 Laser

An Erbium: Glass laser (Megawatt Lasers, 75 joules/pulse) was modified to produce nanosecond pulses by installing an opto-mechanical switch (Taboada Research Instruments, Inc., San Antonio, TX)[11]. The modified laser was used to deliver various pulse energies in the range of 0.28-1.62 J/cm^2 per pulse for a pulse duration range of 42-65 ns. The beam temporal profile can be seen in the figure below. The opto-mechanical switch is set at a 200 Hz angular rate.

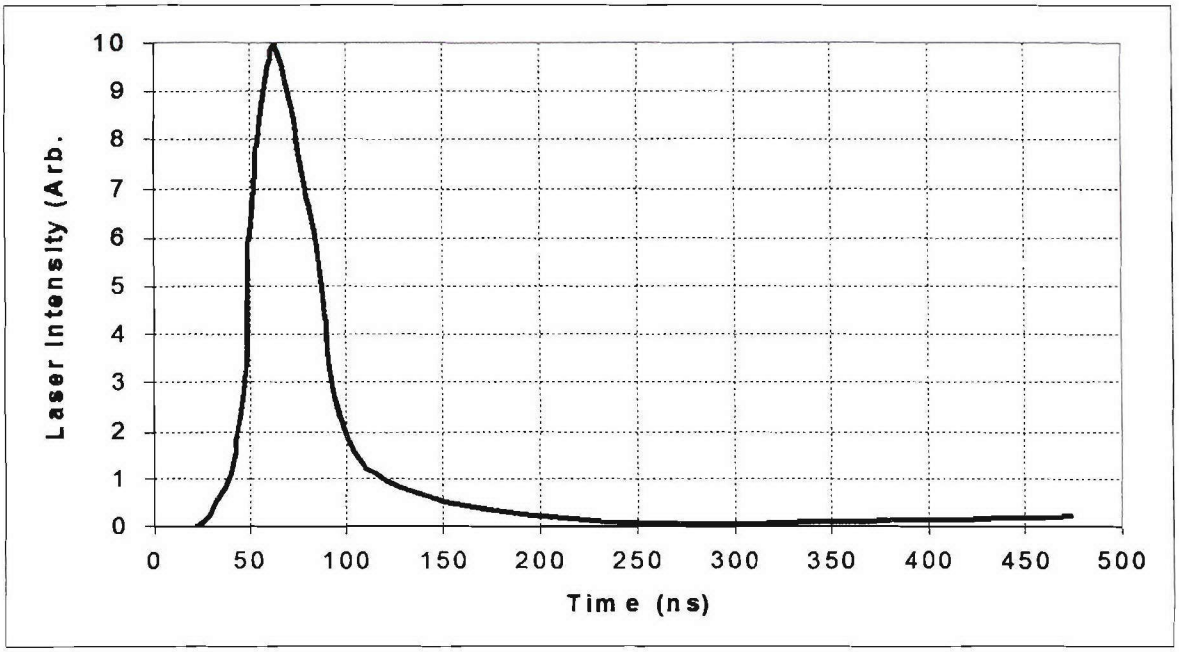

Figure 1. Pulse temporal profile for 1540 nm Er:Glass laser. Opto-mechanical switch set to 200 Hz angular frequency.

Pulse durations were measured by a model ET-3000 InGaAs Electro-Optics Technology, Inc. photodiode connected to at Tektronix model TDS 220 oscilloscope. Energy measurements were made at the location of the beam splitter using an Ophir Laserstar energy meter with Ophir model number: 30(150)-A-HE energy probes. A HeNe laser was used as a sighting beam to locate the exposure point. The Er: Glass laser was mounted with an articulating arm so that exposures were made perpendicular to the subject with the same distance from the focusing lens and the flank skin every time to produce a consistent spot size. The setup is shown in figure 2.

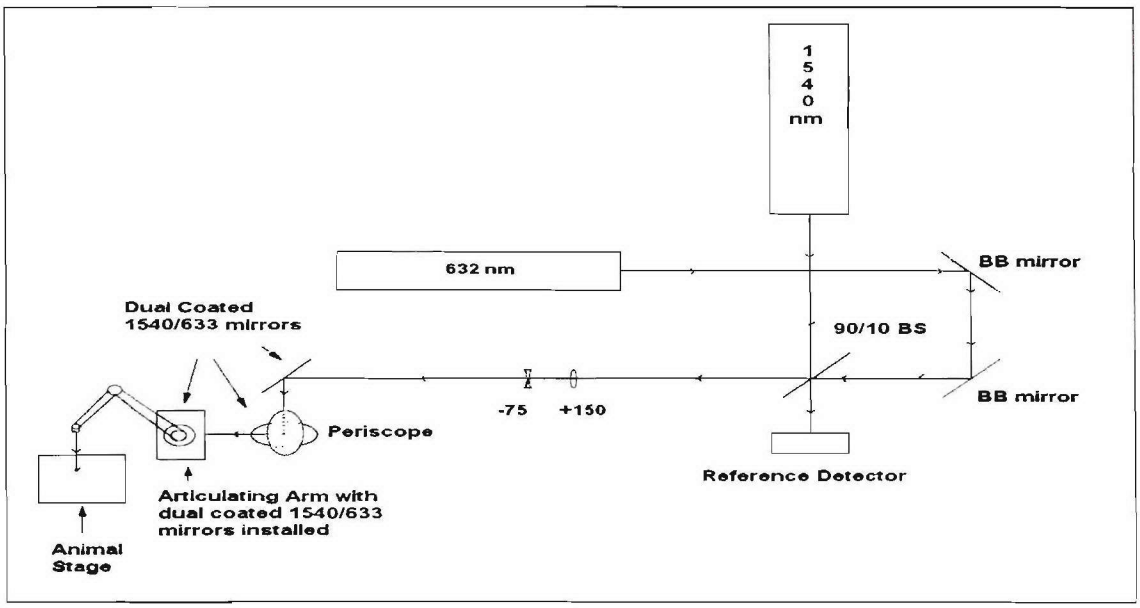

Figure 2. Schematic of the experiment setup. The 632 nm HeNe laser is the sighting beam for the 1540 nm Er: glass laser and was used to help guide 1540 nm pulse delivery. BS: Beam Splitter. BB: Broad Band

The spot size could be varied by changing the distance between the focusing lens at the end of the arm to the exposure site and was adjusted until the $1/e^2$ diameter was 6-mm. The full angle beam divergence was 1.2 mrad in the horizontal direction and 1.10 in the vertical direction. Taking spot size measurements at various points with in the beam path of a 100-cm focal length lens, the M-squared value was calculated to be 4.2 [11]. The beam energy cross section is shown in the figure 3 below.

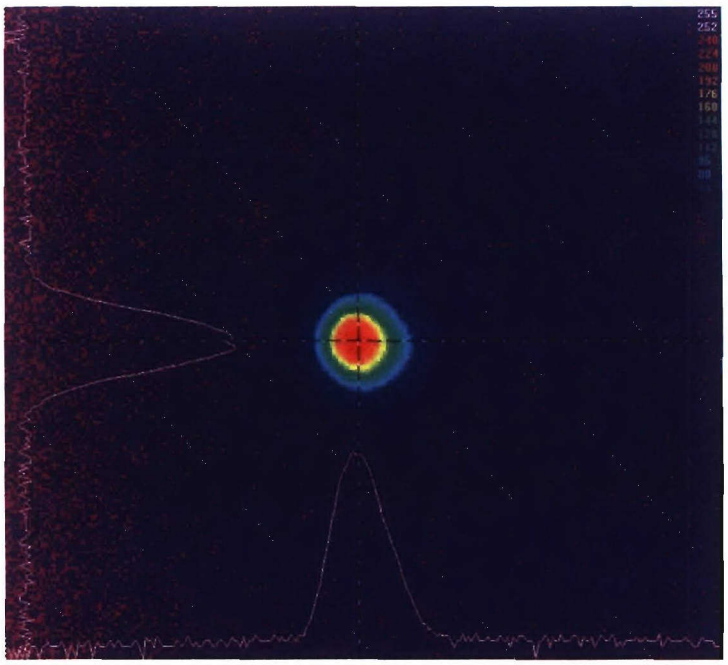

Figure 3. Energy distribution of the beam in the near field.

A metal "aiming ring" was attached to the end of the articulating arm. Exposures were made on each lateral side in a grid pattern consisting of four to six rows and away from the folds of skin near the legs. The number of exposure sites was dependent on the size of the subject on the respective lateral side. Each row consisted of five to six individual exposure locations denoted by 1 cm by 1 cm boxes using black ink marker. Surgical markers were found to smear and interfere with evaluation. Energy delivered was systematically varied for each exposure and randomly delivered at each exposure site. Each subject received a range of 48-56 total exposures for each procedure.

2.3 Evaluations

Three independent evaluations were preformed for each exposure site for the presence of laser-induced skin lesions and then were photographed at 1 and 24 hours. Before a site of exposure was counted as a lesion, at least 2 out of 3 evaluators had to agree a lesion existed. Biopsy specimens were not collected for histological examination.

The cream was diluted to a 1-part cream and 4-parts mineral oil solution for measurement, and demonstrated an absorbance greater than 78 cm^{-1} at 1540-nm when the diffuse reflectance and total transmittance were measured using a single integrating sphere. The undiluted cream was added to evaluate how the minimum visible lesion threshold for a single-pulse laser exposure is changed with the topical agent on the skin. The amount of cream was carefully measured using a needleless syringe, and 0.03 cc were added to each space in the 4 X 7 grid. The topical cream was then carefully smeared with a flat edged tool, and the energy was delivered with randomly-varying levels to each square of one grid. A picture of the cream on skin pre exposure is shown in Figure 4.

Figure 4. 0.03 cc of cream added to each square on the skin and then spread over square area before exposures.

Probit analysis was the statistical method used to determine the estimated dose for a fifty percent probability of producing a lesion, also known as the ED_{50} [12]. Data from each exposure evaluation was input into Probit analysis to calculate the ED_{50} along with their fiducial limits at the 95% confidence level, slopes, and probabilities.

3. EXPERIMENT RESULTS

Erythema was defined as the minimum damage at 1 and 24 hour inspections. The majority of the lesions were of this sort and appeared anywhere from immediately after exposure up to 24 hours later. Lesions close to the threshold at one hour sometimes disappeared after 24 hours or became visible after 24 hours. At the highest energies, immediate whitening of the exposed area surrounded by pink inflammation occurred. The lesions from the higher exposure energies formed scabs on the skin that were present weeks later. The damage can be seen in figure 3. The ED_{50} at 1 and 24 hours for persistent erythema were found to be 2.99 J/cm^2 and 3.04 J/cm^2 respectively. 160 exposures were statistically processed for the ED_{50} at 1 and 24 hour post exposure are shown in tables 1 and 2. The Chi-Square distribution ranged from 0.97 to 1.00 at the 1 hour readings and dropped significantly after 24 hour readings because of insufficient scatter for the probit program. There was consistently damage above a specific exposure level after the 24 hour period. The fiducial limits calculated for all ED_{50} thresholds at both the 1-hour and 24 hour times were within ± 22 percent of the ED_{50} value.

Table 1. MVL-ED_{50} data for a Q-switch pulse duration of 30 ns of 1054 nm laser pulses.

Experimental Setup Number of Subjects & Shots	MVL-ED_{50} (Jcm^{-2}) 1-Hour Reading	MVL-ED_{50} (Jcm^{-2}) 24-Hour Reading	Probit Curve Slope = $\delta p/\delta d$ 24 Hours
6.0-mm diameter spot 3 guinea pigs, 6 flanks, 160 exposures	2.99 (2.7 – 3.3)	3.04 (2.8-3.2)	7.4

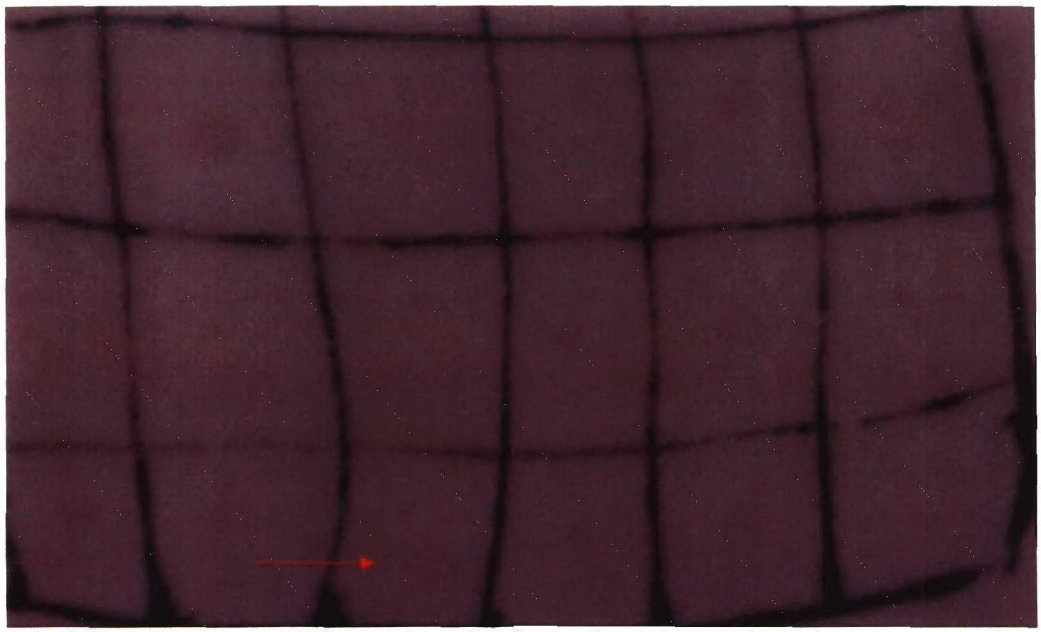

Figure 5. 1540 nm laser exposures after one hour showing erythema at some sites. The arrow points to a lesion.

When laser exposures were made to the covering agent on the skin, a very loud popping sound and a bright stream of light protruded from the exposed surface. Only 28 sites were exposed instead of the 56 originally planned because it was believed extreme thermal damage may have been occurring to the skin surface. The cream was gently removed using baby wipes, and independent evaluations were made of the skin surface for any damage lesions. All three observers agreed that no lesions existed at 1 and 24 hour observations. The cream applied to a chamois to reproduce the loud pops and flashes observed in the experiment. A photo was taken and can be seen in figure 4 below.

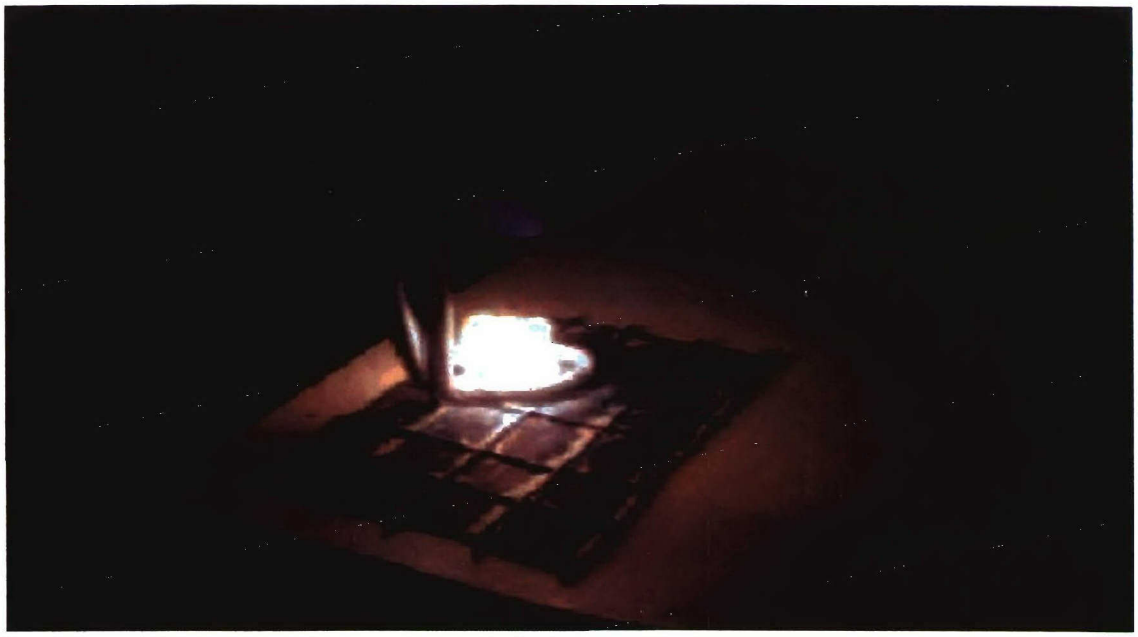

Figure 6. 1540 nm Er: glass laser exposure at 50 ns on cream coated chamois producing a plasma plume. Also seen is the "ring" of the articulating arm for the 1540 nm laser set up.

4. DISCUSSION

Our ED_{50} for a 50 ns pulse at 1.54 μm is presented in our discussion along with a comparison to other reported measurements and a mathematical thermal model. Our results compare very closely with that of Lukashev [1]. They used a 1.2-2 month old 'Big white' pig, which is believed to be a Yorkshire pig, for exposure of 100 ns at 1540 nm for a 2.5-3.5 mm diameter. The ED_{50} was found to be 3.0 ± 1.1 J/cm² and 3.5 J/cm² for 1 and 24 hour post exposure respectively. They reported no dependency between ED_{50} and laser beam spot size for beam diameters between 2-10 mm [1]. Cain reported an ED_{50} of 6.3 J/cm² and 6.1 J/cm² for a Yucatan minipig at 1 hour and 24 post exposure for 31 ns at 1540 nm for a 5 mm spot size [2]. These results are close to the results for the guinea pig. Table 2 shows the comparison.

Table 2. Comparison of ED50 values for Q-switched 1540 nm laser at various pulse widths and spots

Experimental Setup Number of exposures & shots Animal Mode	**MVL-ED_{50} (Jcm^{-2}) 1-Hour Reading**	**MVL-ED_{50} (Jcm^{-2}) 24-Hour Reading**
5.0-mm diameter spot 30 ns 216 exposures (Cain) Porcine	6.3	6.1
3.5 -mm beam diameter 100 ns 266 exposures (Lukashev) Porcine	3.2	3.1
6.0-mm diameter spot 160 exposures Guinea Pig (Cavia Porcellus)	3.0	3.0

The ANSI (Z136.1-2000) gives the Maximum Permissible Exposure (MPE) to 1540 nm for the experimental parameters used for in study to be 1 J/cm^2 [7]. Our findings are consistent with the standard and are above the MPE. Other studies for damage evaluation of lasers down to the cellular level using guinea pigs have been done at 355, 532, 694, and 1064 nm [12-14]. The damage evaluation procedures described in the papers are different than the procedure described in this paper, but the responses to laser exposures are similar.

Loud "pops" and mini flashes of light, especially at the higher energies, occurred during the ED_{50} experiments. For each exposure, the sounds and light flashes that were observed were noted for each respective exposure parameter. The ED_{50} for 1 and 24 hours did not change much, and the data suggests that some of the lesions close to the threshold that were either visible or undetectable at 1 hour became the opposite at 24 hours. Lesions produced by the highest energies remained for weeks after exposure. It was suspected that some of the damage may have been attributed more to photoacoustic effects than thermal effects because of the "pops" and flashes of light. Part of the discussion will include an explanation of the thermal damage model that was used to help determine the damage mechanism.

4.1 Thermal Model

The model employed to estimate temperature effects along with the evaluation of thresholds for tissue damage was a validated legacy model, commonly referred to as the "Takata Skin Model" [15]. The Takata Model is a time-dependent finite-difference method solution of the two-dimensional (cylindrical symmetry) bio-heat equation. Features of the model include a user-configurable multi-layer tissue model. Thermo-mechanical as well as optical properties of the tissue layers are user inputs. Laser parameters are also user configurable, and sources may include a multitude of single-wavelength sources. The spectrum for a broad band source, which is not specified as an input, can be given as a computed black body distribution corresponding to a known color temperature. Spatial profiles may be flat top, Gaussian, annular, or user defined. Single or multiple pulses or the temporal behavior defined by the user for each point in time may be selected for the temporal profile. The geometric model of beam irradiance is employed along with linear absorption of the tissue to estimate energy deposition rates at various points within the computational grid. Boundary conditions include constant flux surface convection at the tissue-air interface. Thermal effects of variable blood flow with tissue depth are evaluated. Phase change of the water content of the tissue as well as increased absorption for charred tissues are evaluated through empirical relationships. The model does not incorporate tissue optical scattering effects.

For all runs, the model determines an adaptive time step which captures rapid changes in temperature at high time resolution. The adaptive time step also provides for large time steps in regions for which there is a "steady state" or little change in the temperature distribution. The minimum and maximum coordinates for the grid along with the grid point spacing is defined by the user. The Takata model execution results in a time-temperature history at each point within the computational grid. Each point within the grid is evaluated for potential damage over the duration of the simulation through an

Arrhenius damage integral, shown in equation (1), with temperature dependent damage rate coefficients. The damage integral is normalized against experimental data for first-degree through third-degree burns. Henriques set up the rate equation such that a first-degree burn is represented by a damage integral value of 0.1 and second-degree burn is represented by a value of 1.0 [16].

$$\Omega(r,z) = A\int_0^t \exp(-E/RT)dt \qquad (1)$$

where A is the pre-exponential factor (s^{-1}), E is activation energy, R=2.0 cal/(MK) is the universal gas constant, T is the absolute temperature of a given coordinate in time, T(z,r,t), and t is the time at final recovery of temperature after exposure.
The variable A is a normalized constant and E is the activation energy for a reactive process leading to damage. The values for each are respectively given as:

$A = 3.1 \times 10^{98}$ (1/s) $\qquad 317 < T < 323$ K
$E = 628{,}000$ (cal/M).

The values are taken from the work of Henriques for controlled temperature exposure on skin [16]. Critical parameters within the thermal model are the absorption coefficients as a function of wavelength. There is limited data for absorption coefficients of skin in the infrared range, and the greatest sources of uncertainty are our absorption coefficient parameters. There is limited absorption coefficient data in the infrared region. The absorption coefficient values for 1500-1550 range from ~ 1.5 cm^{-1} to 15 cm^{-1} for human and Yucatan mini-pig skin [17].

4.2 Model Results

An absorption coefficient of 8 c m^{-1} for porcine at 1500 nm provided by Du was used [18]. The model predicted that the ED_{50} pulse power produced no damage and increased the surface temperature to 4.5 $C°$. Lukashev also used a model to predict temperature increases and received a temperature of 8.0 $C°$ at 1540 nm for nanosecond pulses [19]. No damage was achieved in the model until the pulse power was increased to three times larger than the ED_{50} value of power. At this pulse energy, the temperature rise to create a second degree burn was 22.3C°. An absorption coefficient of 1.5 cm^{-1} for human skin measured in-vitro was also used, and a similar result was observed [17]. A second layer was added to emulate the epidermal and dermal layers of skin with respective absorption coefficients obtained from Cain without any success of lowering the pulse power needed to produce damage[20]. The epidermal and dermal layers are considered homogenous and of "infinite thickness", providing a solution for axial boundaries at which little energy is conducted within the simulation time. The coefficient values and predicted temperature rises are shown in table 3.

Table 3. Optical properties of skin at 1540 nm and model predictions for temperature rise on skin surface. The dashes under the dermal column denote one layer used in the model.

Skin Type	Wavelength (nm)	Epidermis μ_a (1/cm)	Dermis μ_a (1/cm)	Predicted Temp Rise (C°)
Yucatan Mini Pig	1500	8	--	4.5
Yucatan Mini Pig	1540	6	5.42	3.5
Human (in-vitro)	1550	1.5	--	1.1

It has been determined that a single pulse of at our given energy and duration was within thermal and stress confinement and that the pulse duration was much shorter than relaxation time of the tissue [21]. This suggests that the damage mechanism is not entirely attributed to photothermal interactions as much as thermomechanical interaction. To see if thermomechanical interactions were at play, laser induced breakdown thresholds (LIB) were looked at. Our calculated incident irradiance of 9.7×10^7 W/cm² was very close to the plasma threshold of 10^8 W/cm² in the presence of local impurities such as dead skin [21]. This suggests that plasma formation was likely and would help to explain the "pops" and flashes of light seen at exposure sites. To find out if the threshold for laser induced breakdown had been exceeded, the electric field intensity is given by equation 2:

$$E = \left(\frac{2\Phi}{cn\varepsilon_0} \right)^{1/2} \qquad (2)$$

where Φ is the power density, ε_0 is the permittivity of free space, c is velocity of light, and n is the refractive index [22]. The index of refraction for hydrated stratum corneum is 1.41 and was used [23]. The calculated electric field intensity was 2.28×10^7 V/m and was enough to cause LIB [22].

It is believed that plasma had been generated via an adiabatic process and that it created a shield by absorbing the incident radiant energy and prevented some of the energy from being deposited in the skin. Any damage that occurred had resulted from acoustic and shock waves from the plasma as well as the high plasma temperature which can be as greater than 10,000 K [24].

When the skin was coated with the cream, it was noted that a loud popping noise and an intense flame plume approximately ~ 5-8 cm in height occurred when the laser exposures were delivered. After exposures, the paint was gently wiped off using baby wipes. Photos were taken of the skin after the paint was removed, and exposure sites were inspected by three evaluators for any lesions. All three evaluators agreed no lesions existed at 1 and 24 hour inspections, even at the highest energy of 5.62 J/cm². The conclusion was that because paint had been highly absorbing in the near infrared, it caused ionization and induced an electron avalanche via an adiabatic process. Because the absorption coefficient of the plasma is much greater than the covering agent, nearly all of the incident energy had been absorbed by the plasma and prevented any appreciable

penetration into the skin, thus the effect was plasma shielding. The expectation had been that lesions would still exist because of acoustic effects produced by the intense plasma. Since no lesions existed, it was questioned whether thermomechanical effects had helped to generate the lesions on the skin for the ED_{50} determination or if it should be attributed more to the thermal effects of the plasma or other unaccounted phenomena. More studies should be done to help clarify the damage mechanism.

5. CONCLUSION

In this study we experimentally determined the reaction of guinea pig skin (in vivo) to 1540 nm radiation of an Er: Glass laser for 30 ns pulses at a spot size of 6 mm diameter. The ED_{50} value was found to be 3.0 J/cm² and 3.04 J/cm² for 1 and 24 hours respectively and was above the MPE of 1 J/cm^2 as given by the ANSI (Z136.1-2000) [7]. A cream added to the skin and exposed at the same energies used to determine the ED_{50} prevented all damage at those energies because of plasma shielding thus increasing the ED_{50} for 1540 nm using 42-65 ns pulses. The cream protected the skin for all the available pulse energies and could serve as a skin protectant for similar skin exposures. When we compare our results to similar studies using porcine, the ED_{50} values are close suggesting that guinea pigs may be a suitable model for laser exposure studies. Thermal modeling using the Takata skin model of the experiment parameters at the ED_{50} threshold revealed that the damage induced on the skin for the experiments did not match the predicted damage and temperature rise on the skin surface. The predicted temperatures were too low to cause the observed ED_{50}s and may be attributed to unaccounted for heat or photoacoustic and shock waves from plasma formation on the skin. More research is needed to clarify the damage mechanism at short pulse and peak irradiances of *in vivo* subjects.

6. REFERENCES

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APPENDIX A

GP-1 1-HR EVALUATIONS

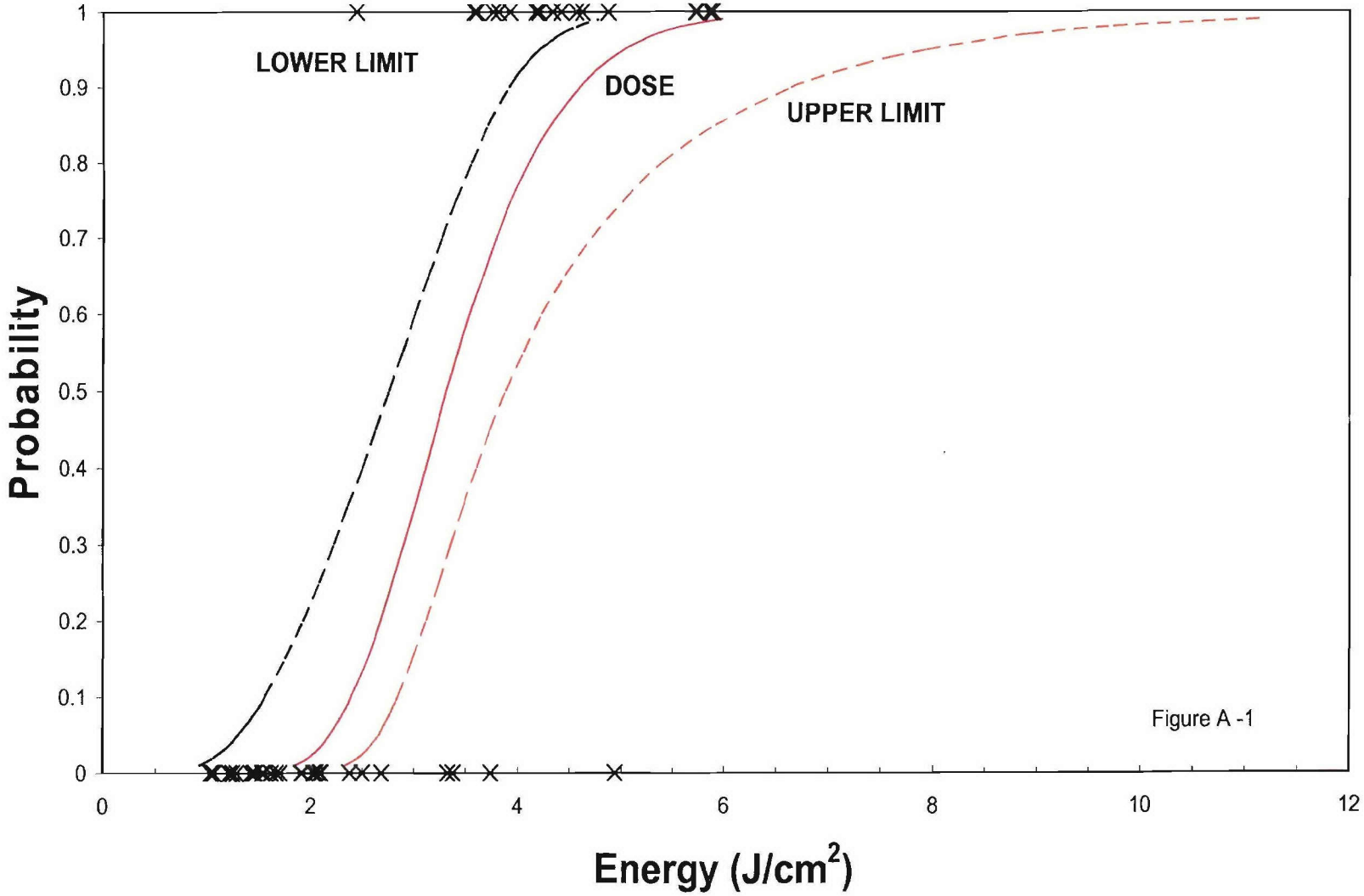

Figure A -1

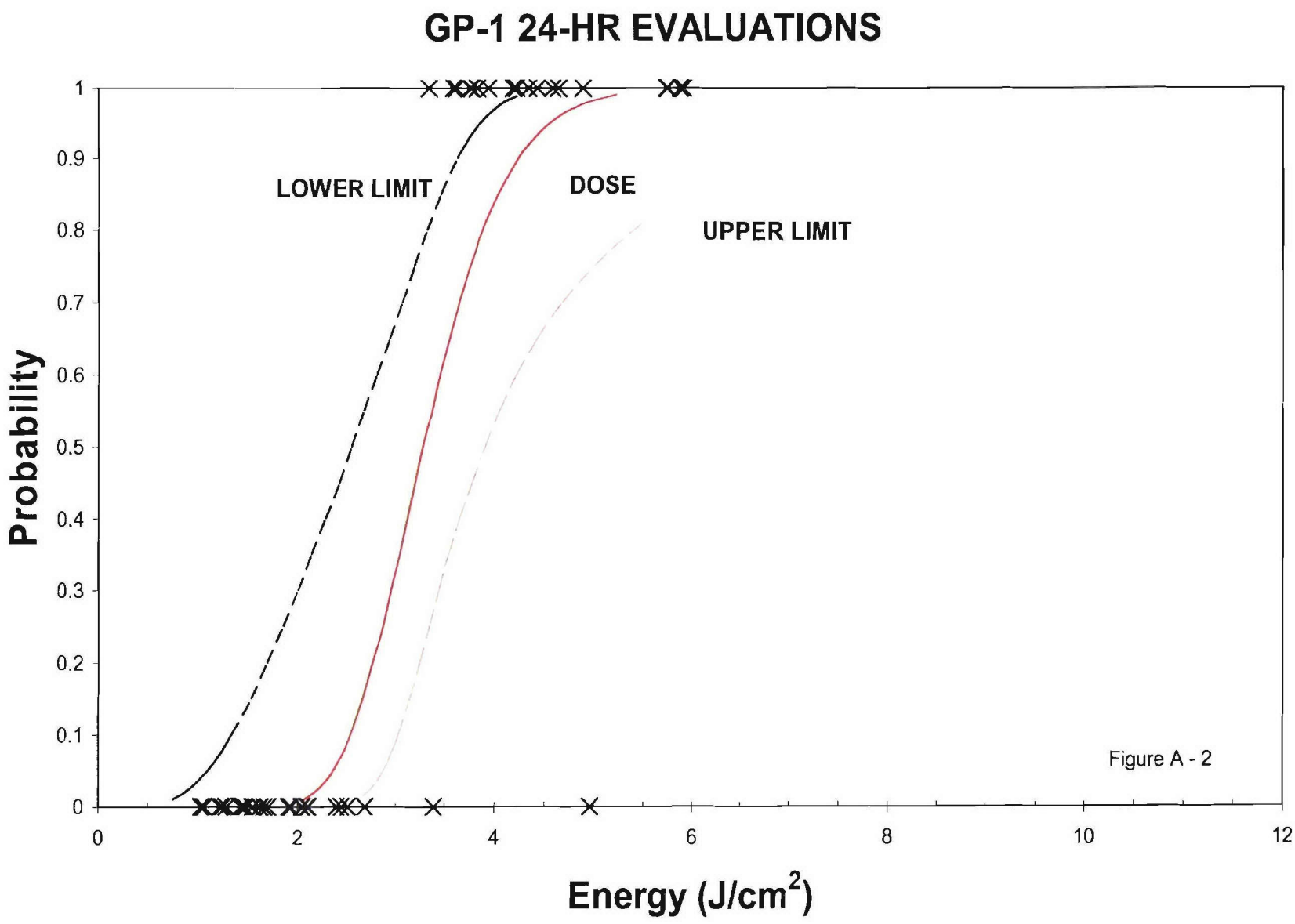
GP-1 24-HR EVALUATIONS
LOWER LIMIT
DOSE
UPPER LIMIT
Probability
Energy (J/cm2)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
2
4
6
8
10
12

Figure A - 2

GP-6 1-HR EVALUATIONS

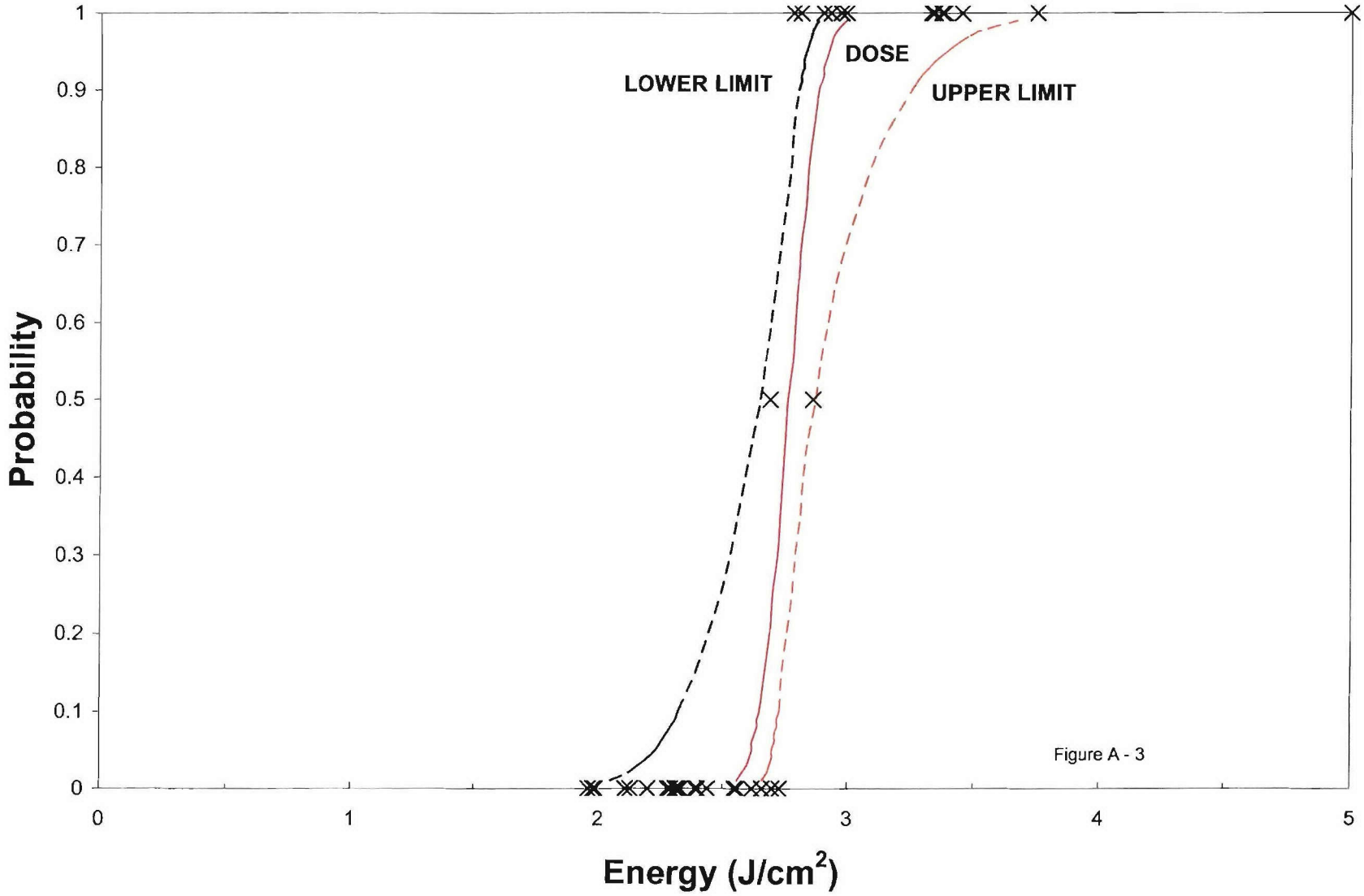

Figure A - 3

GP-6 24-HR EVALUATIONS

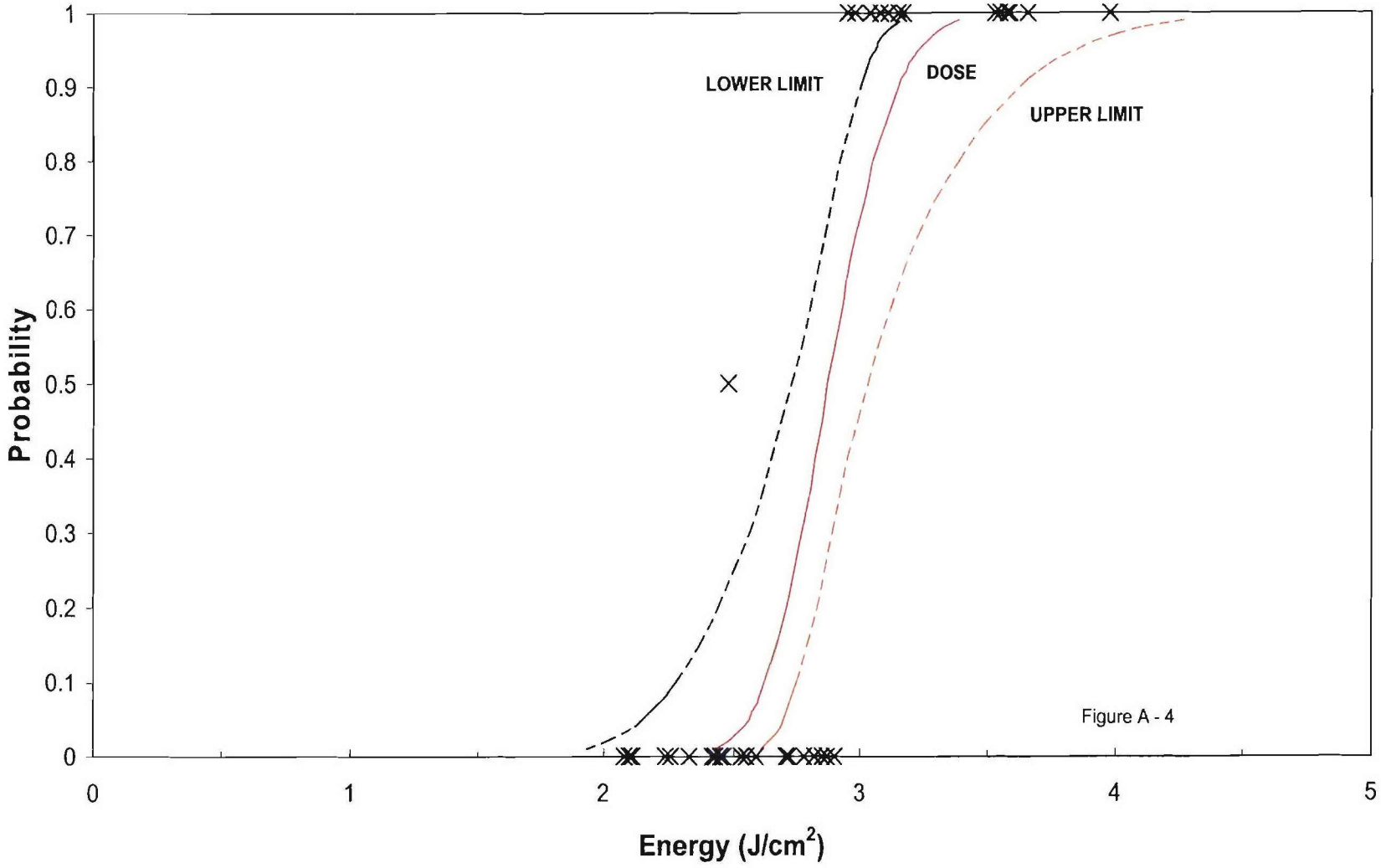

Figure A - 4

GP-7 1-HR EVALUATIONS

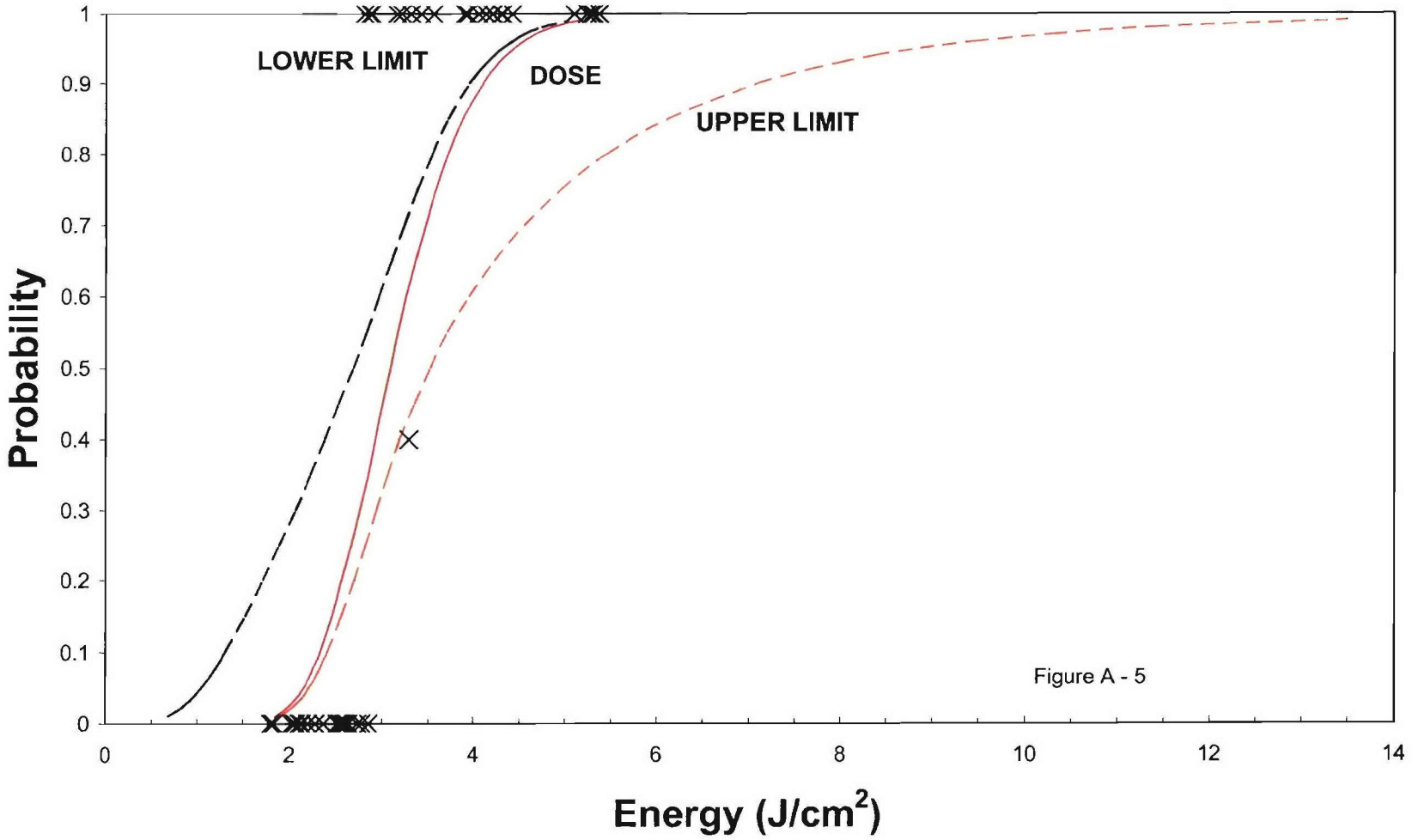

Figure A - 5

GP-7 24-HR EVALUATIONS

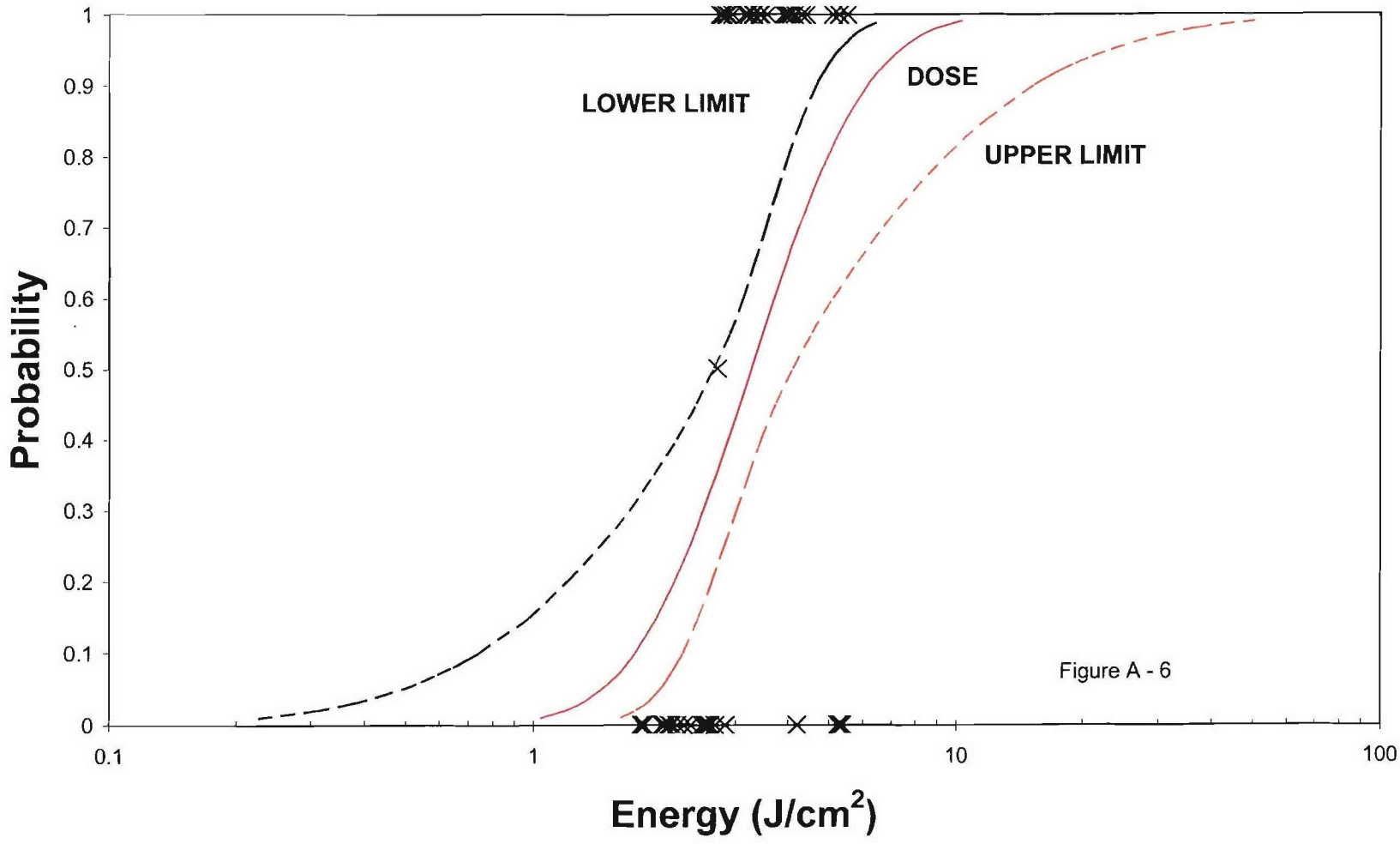

Figure A - 6

COMBINED 1-HR EVALUATIONS

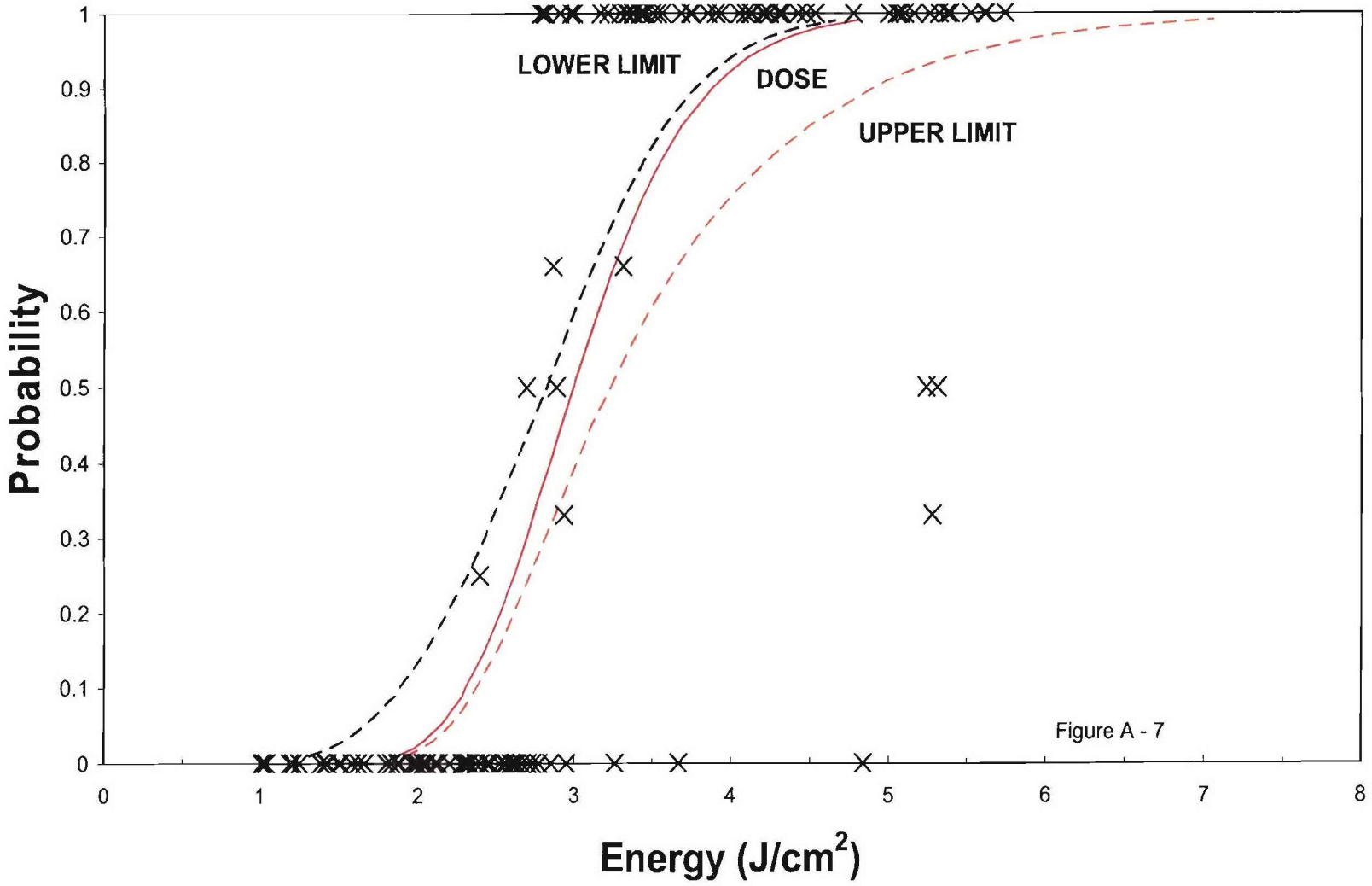

Figure A - 7

COMBINED 24-HR EVALUATIONS

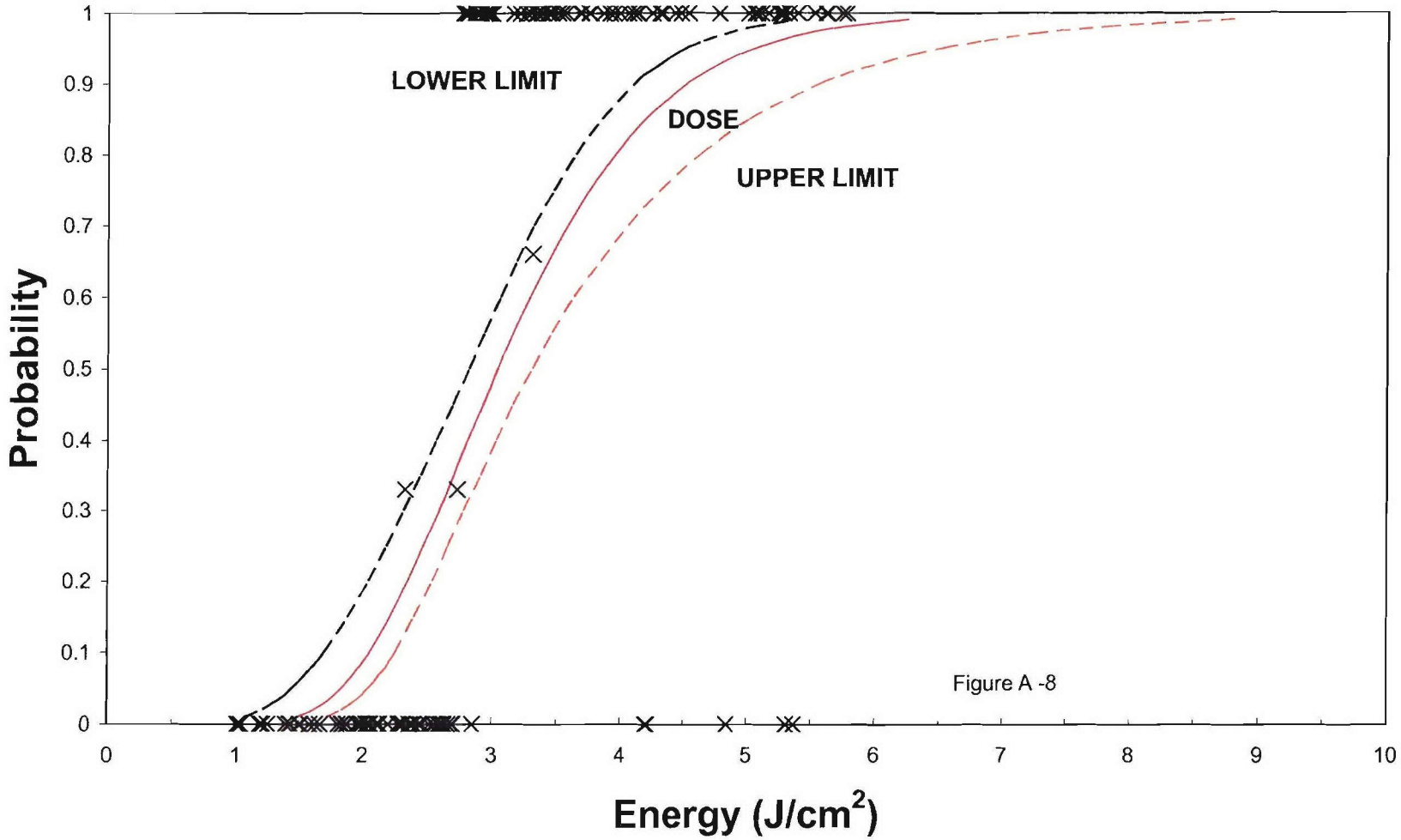

Figure A -8